

THERAPEUTIC AND DIAGNOSTIC COMPOSITIONSJno. 7
a/aFIELD of the Invention

The present invention relates generally to therapeutic compositions for the treatment and/or prophylaxis of intestinal disease conditions in animals and birds caused or exacerbated by *Lawsonia intracellularis* or similar or otherwise related microorganism. The present invention also contemplates methods for the treatment and/or prophylaxis of such intestinal disease conditions and to diagnostic agents and procedures for detecting *Lawsonia intracellularis* or similar or otherwise related microorganism.

a 10 Background of the Invention

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOS.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

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Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

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The meat industry in Australia and, indeed, in most countries of the world, is an important aspect of the overall livestock industry. However, the meat industry is subject to rapid economic downturn in response to disease conditions affecting the animals as well as human diseases putatively carried by the animals. It is important, therefore, to have well defined treatment, prophylactic and diagnostic procedures available to deal with infections or potential infections in animals and humans.

Pigs form a major component of the meat industry. However, pigs are sensitive to a wide spectrum of intestinal diseases collectively referred to as porcine proliferative enteropathy (PPE). This disease has previously been known as intestinal adenomatosis complex (1).

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porcine intestinal adenomatosis (PIA), necrotic enteritis (2), proliferative haemorrhagic enteropathy (3), regional ileitis (4), haemorrhagic bowel syndrome (5), porcine proliferative enteritis and *Campylobacter* spp induced enteritis (6).

5 There are two main forms of PPE: a non-haemorrhagic form represented by intestinal adenomatosis which frequently causes growth retardation and mild diarrhoea; and a haemorrhagic form, which is often fatal, represented by proliferative haemorrhagic enteropathy (PHE) where the distal small intestine lumen becomes engorged with blood. PPE has been reported in a number of animal species including pigs (14), hamsters (7), ferrets (15), guinea 10 pigs (16), rabbits (17) as well as avian species (18).

The causative organism of PPE is a *Campylobacter*-like organism referred to herein as "Lawsonia intracellularis" (26). The organism has also been previously referred to as *Ileal symbiont intracellularis* (7). PPE-like diseases in pigs may also be caused by other pathogens 15 such as various species of *Campylobacter* (8).

Lawsonia intracellularis is an intracellular, possibly obligate intracellular, bacterium. It can only be cultured *in vitro* with tissue culture cells (9, 26). Pigs suffering from PPE are characterised by multiple abnormal immature crypts and *L. intracellularis* is located in the 20 cytoplasm of these crypt cells.

PPE is a significant cost component associated with the pig industry, especially in terms of stock losses, medication costs, reduced growth rates of pigs and increased feed costs. PPE also contributes to downstream indirect costs in, for example, additional labour costs and 25 environmental costs in dealing with antibiotic residue contamination and in control measures to prevent the organism being passed on or carried to other animals or humans.

Current control strategies for PPE rely on the use of antibiotics. However, such a strategy is considered to be short to medium term especially as governmental regulatory pressures tend to 30 target animal husbandry practices which are only supported by prophylactic antibiotics. There

is a need, therefore, to develop effective, safe and low cost alternatives to the use of antibiotics. There is also a need to extend this alternative to antibiotics to similar organisms which infect other animals such as humans.

5 In work leading up to the present invention, the inventors sought to develop vaccines for the prophylaxis and treatment of PPE in animals and birds. The vaccines of the present invention provide an efficacious alternative to the use of antibiotics with a range of consequential husbandry and medical benefits.

Summary of the Invention

10 Accordingly, one aspect of the present invention provides a vaccine composition for the prophylaxis or treatment of infection in an animal or bird by *L. intracellularis* or similar or otherwise related microorganism, said vaccine composition comprising an immunogenic, non-pathogenic form of *L. intracellularis* or related microorganism or an immunogenic component thereof and one or more carriers, diluents and/or adjuvants suitable for veterinary or
15 pharmaceutical use.

The present invention is particularly useful and is exemplified hereinafter in relation to the protection and/or treatment of pigs from infection with *L. intracellularis*. However, this is done with the understanding that the present invention extends to the prophylaxis and treatment of
20 all animals including humans and birds from infection with *L. intracellularis* and/or related microorganisms. Animals contemplated by the present invention include but are not limited to humans, primates, companion animals (e.g. cats, dogs), livestock animals (e.g. pigs, sheep, cattle, horses, donkeys, goats), laboratory test animals (e.g. mice, rats, guinea pigs, rabbits) and captive wild animals (e.g. kangaroos, foxes, deer). The present invention also extends to birds
25 such as poultry birds, game birds and caged birds.

Furthermore, the present invention extends to all isolates and sub-types of *L. intracellularis* as well as other species of the genus *Lawsonia* or other microorganisms related thereto at the nucleotide, biochemical, structural, physiological and/or immunointeractive level. Reference
30 hereinafter to "*Lawsonia intracellularis*" or its abbreviation "*L. intracellularis*" includes all

microorganisms similar to or otherwise related to this microorganism. For example, a related microorganism may have a nucleotide sequence similarity at the chromosome or extrachromosomal level of at least about 60%, more preferably at least about 70% and even more preferably greater than at least about 80% with respect to all or part of a nucleotide sequence within the chromosome or extrachromosomal elements of *L. intracellularis*. For example, these percentage similarities may relate to the sequence set forth in SEQ ID NO:5. This sequence is a portion of the *L. intracellularis* chromosome.

Accordingly, this aspect of the present invention is directed to a vaccine composition for the prophylaxis and/or treatment of infection in a pig by *L. intracellularis*, said vaccine composition comprising an immunogenic, non-pathogenic form of *L. intracellularis* or related microorganism or an immunogenic component thereof and one or more carriers, diluents and/or adjuvants suitable for veterinary or pharmaceutical use.

15 The term "immunogenic component" refers to *L. intracellularis* (in attenuated non-pathogenic or killed form) or a component of *L. intracellularis* including a peptide, polypeptide or a protein encoded by DNA from or derived from *L. intracellularis* which is capable of inducing a protective immune response in a pig. A protective immune response may be at the humoural and/or cellular level and generally results in a substantial reduction in the symptoms of PPE in 20 pigs. The vaccine compositions will comprise an effective amount of immunogenic component such as to permit induction of a protective immune response.

According to this aspect of the present invention there is provided a vaccine composition for the prophylaxis and treatment of a pig by *L. intracellularis*, said vaccine composition comprising an amount of at least one immunogenic component from *L. intracellularis* or related microorganism effective to induce a protective immune response in said pig against *L. intracellularis* or related microorganism, said vaccine composition further comprising one or more carriers, adjuvants and/or diluents suitable for veterinary or pharmaceutical use.

30 The immunogenic component may be a naturally occurring peptide, polypeptide or protein, a

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carbohydrate, lipid or nucleic acid (e.g. DNA) or any combination thereof isolated from *L. intracellularis* or a cell culture thereof or a recombinant form of a peptide, polypeptide or protein encoded by DNA from or derived from *L. intracellularis* or is a derivative of said peptide, polypeptide or protein.

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An isolated component of *L. intracellularis* is a component which has undergone at least one purification step or which has undergone at least partial concentration from a cell culture comprising *L. intracellularis* or from a lysed preparation of *L. intracellularis* cells. The purity of such a component from *L. intracellularis* which has the requisite immunogenic properties 10 is preferably at least about 40%, more preferably at least about 50%, even more preferably at least about 60%, still more preferably at least about 70% and even more preferably at least about 80-90% or greater relative to other components in a preparation as determined by molecular weight, immunogenic activity or other suitable means.

15 A particularly useful form of the vaccine is a whole cell vaccine which comprises *L. intracellularis* in attenuated or otherwise non-pathogenic form or killed cells or various fractions thereof.

Attenuated or non-pathogenic cells include killed *L. intracellularis* cells prepared, for example, 20 by heat, formalin or other chemical treatment, electric shock or pressure and such cells are particularly useful in the practice of the present invention.

According to this aspect of the present invention there is provided a vaccine composition for the prophylaxis and/or treatment of infection in a pig by *L. intracellularis* or related 25 microorganism said vaccine composition comprising a killed preparation of *L. intracellularis* or related microorganism or an immunogenic fraction thereof and one or more carriers, diluents and/or adjuvants suitable for veterinary or pharmaceutical use.

In an alternative embodiment, a recombinant vaccine may be employed. The recombinant 30 vaccine may comprise one or more recombinant peptides, polypeptides or proteins derived from

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L. intracellularis or is a recombinant molecule immunologically related to a peptide, polypeptide or protein derived from *L. intracellularis* or may be a fusion molecule having a first portion comprising a peptide, polypeptide or protein derived from *L. intracellularis* and a second heterologous peptide, polypeptide or protein which may be useful, for example, as a 5 carrier molecule or an adjuvant or an immune stimulating molecule such as cytokine. A particularly useful recombinant protein from *L. intracellularis* comprises a peptide, polypeptide or protein derived from the cell surface or membrane of *L. intracellularis*, is an enzyme in a metabolic pathway within *L. intracellularis* or is a refolding and/or heatshock protein. In a preferred embodiment, the protein is a refolding/heatshock protein such as but not limited to 10 GroEL and GroES. Other putative vaccine candidates include flagellar basal body rod protein, S-adenosylmethionine: tRNA ribosyltransferase-isomerase, enoyl-(acyl-carrier-protein) reductase, N-acetyl muramoyl-L-alanine amidase (autolysin), UOP-3-0-[3-hydroxymyristoyl] glucosamine N-acetyltransferase and a glucarate transporter.

15 According to a preferred embodiment, the present invention relates to a vaccine composition for the prophylaxis and/or treatment of infection in a pig by *L. intracellularis* or related microorganism, said vaccine composition comprising at least one recombinant peptide, polypeptide or protein from *L. intracellularis* and wherein said recombinant peptide, polypeptide or protein is capable of inducing a protective immune response against *L. 20 intracellularis* in pigs, the vaccine composition further comprising one or more carriers, diluents and/or adjuvants suitable for veterinary or pharmaceutical use.

In a particularly preferred embodiment, the recombinant protein is GroEL having an amino acid sequence as set forth in SEQ ID NO:2 or is a protein having a predicted amino acid sequence 25 with at least about 40%, at least about 60%, or more preferably at least about 70% and even more preferably at least about 80-90% or greater similarity to all or part of the amino acid sequence set forth in SEQ ID NO:2.

In another embodiment, the recombinant molecule is GroES having an amino acid sequence as 30 set forth in SEQ ID NO:4 or is a molecule having an amino acid sequence at least about 40%,

at least about 60%, more preferably at least about 70% and even more preferably at least about 80-90% or greater similarity to all or part of the amino acid sequence set forth in SEQ ID NO:4.

Another embodiment of the present invention includes and comprises a peptide, polypeptide 5 or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:1 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

10 In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:3 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

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In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:5 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related

20 microorganism.

In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:6 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and 25 which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:~~8~~ or having at least 30 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and

which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

In a related embodiment, the present invention includes and comprises a peptide, polypeptide 5 or protein encoded by a nucleotide sequence as set forth in SEQ ID NO: ¹² ~~11~~ or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

10 In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO: ¹⁵ ~~13~~ or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

15 In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO: ²¹ ~~15~~ or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related 20 microorganism.

In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO: ²⁸ ~~17~~ or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and 25 which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO: ³⁹ ~~18~~ or having at least 30 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and

which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

B In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO: ³⁰19 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

10 In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO: ³¹20 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

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B In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO: ³²21 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related 20 microorganism.

B In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO: ³³22 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and 25 which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

B In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO: ³⁴23 or having at least 30 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and

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which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

Preferred percentage similarities include at least about 50% or at least about 60% or at least 5 about 70-90%.

Ans 27
Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions.

10 Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and 15 from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

The present invention also contemplates peptides, polypeptides or proteins having an amino acid sequence substantially as set forth in one of SEQ ID NO:7 or 9 or 10 or 12 or 14 or 16 or 20 having at least 40% similarity thereof or to all or part thereof. Preferred percentage similarities include at least about 50%, or at least about 60% or at least about 70-90%.

The present invention further extends to a vaccine comprising a recombinant vaccine vector encoding a peptide, polypeptide or protein derived from *L. intracellularis* or related 25 microorganism as described above. The vaccine vector may be of viral, yeast or bacterial origin and would be capable of expression of a genetic sequence encoding a peptide, polypeptide or protein from *L. intracellularis* in a manner effective to induce a protective immune response. For example, a non-pathogenic bacterium could be prepared containing a recombinant sequence capable of encoding a peptide, polypeptide or protein from *L. intracellularis*. The recombinant 30 sequence would be in the form of an expression vector under the control of a constitutive or

inducible promoter. The bacterium would then be permitted to colonise suitable locations in a pig's gut and would be permitted to grow and produce the recombinant peptide, polypeptide or protein in amount sufficient to induce a protective immune response against *L. intracellularis*.

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In a further alternative embodiment, the vaccine may be a DNA vaccine comprising a DNA molecule encoding a peptide, polypeptide or protein from *L. intracellularis* and which is injected into muscular tissue or other suitable tissue in a pig under conditions sufficient to permit transient expression of said DNA to produce an amount of peptide, polypeptide or 10 protein effective to induce a protective immune response.

The vaccines of the present invention may contain a single peptide, polypeptide or protein or a range of peptides, polypeptides or proteins covering different or similar epitopes. In addition, or alternatively, a single polypeptide may be provided with multiple epitopes. The latter type 15 of vaccine is referred to as a polyvalent vaccine. A multiple epitope includes two or more repeating epitopes.

The formation of vaccines is generally known in the art and reference can conveniently be made to Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pennsylvania, 20 USA.

The present invention, therefore, contemplates a pharmaceutical composition or vaccine composition comprising an immunity developing effective amount of one or more of:

- 25 (i) an immunogenic component from *L. intracellularis*;
- (ii) a recombinant peptide, polypeptide or protein from *L. intracellularis* having immunogenic properties; and/or
- (iii) whole cells or a component or fraction thereof from *L. intracellularis*.

30 The above components are referred to hereinafter as "active ingredients". The active

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ingredients of a vaccine composition as contemplated herein exhibit excellent therapeutic activity, for example, in the treatment and/or prophylaxis of PPE when administered in an amount which depends on the particular case. For example, for recombinant molecules, from about 0.5 μ g to about 20 mg may be administered. Other useful effective amounts include 1 5 μ g to about 10 mg, 10 μ g to about 5 mg and 50 μ g to about 1 mg. The important feature is to administer sufficient to induce an effective protective immune response. The above amounts may be administered as stated or may be calculated per kilogram of body weight. Dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated 10 by the exigencies of the therapeutic situation. Booster administration may also be required.

The active ingredients may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intramuscular, subcutaneous, intranasal, intradermal or suppository routes or implanting (eg using slow release technology). Depending on the route 15 of administration, the active ingredients which comprise, for example, peptides, polypeptides or proteins may be required to be coated in a material to protect said ingredients from the action of enzymes, acids and other natural conditions which may inactivate said ingredients.

The term "adjuvant" is used in its broadest sense and includes any immune stimulating 20 compound such as interferon. Adjuvants contemplated herein include resorcinols, non-ionic surfactants such as polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether and Freund's complete and incomplete adjuvant.

The active compounds may also be administered parenterally or intraperitoneally. Dispersions 25 can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where 30 water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile

injectable solutions or dispersion. In all cases the form must be fluid to the extent that easy syringability exists unless the pharmaceutical form is a solid or semi-solid such as when slow release technology is employed. In any event, it must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of 5 microorganisms.

The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for 10 example, by the use of a coating such as licithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. 15 Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as 20 required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a 25 powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

Carriers and diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media 30 and agents in vaccines is well known in the art. Except insofar as any conventional media or

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agent is incompatible with an active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

5 Still another aspect of the present invention is directed to antibodies to the peptides, polypeptides or proteins from *L. intracellularis* or recombinant forms thereof or non-proteinaceous molecules such as carbohydrates. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to *L. intracellularis* or may be specifically raised to specific molecules or whole cells or components or fractions thereof.

10 The antibodies of the present invention are particularly useful for immunotherapy and vaccination and may also be used as a diagnostic tool for infection or for monitoring the progress of a vaccination or therapeutic regime.

For example, recombinant *L. intracellularis* peptides, polypeptides or proteins can be used to 15 screen for naturally occurring antibodies to *L. intracellularis*. Alternatively, specific antibodies can be used to screen for *L. intracellularis*. Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA. Hereinafter, an immunogenic component is considered to encompass an immunogenic component of *L. intracellularis* and includes recombinant molecules, whole cells and cell extracts.

20 In accordance with this aspect of the present invention, the immunogenic components are particularly useful in screening for antibodies to *L. intracellularis* and, hence, provide a diagnostic protocol for detecting *L. intracellularis* infection. Alternatively, biological samples can be directly screened for *L. intracellularis* using antibodies raised to immunogenic 25 components.

Accordingly, there is provided a method for the diagnosis of *L. intracellularis* infection in a pig comprising contacting a biological sample from said pig with an immunogenic component binding effective amount of an antibody for a time and under conditions sufficient for an 30 immunogenic component-antibody complex to form, and then detecting said complex.

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The presence of immunogenic components (or antibodies thereto) in a pig's blood, serum, or other bodily fluid, can be detected using a wide range of immunoassay techniques such as those described in US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. This includes both single-site and two-site, or "sandwich", assays of the non-competitive types, as well as in the traditional 5 competitive binding assays. Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention.

- 10 Briefly, in a typical forward assay, an immunogenic component-specific antibody is immobilised onto a solid substrate to form a first complex and the sample to be tested for immunogenic component brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-immunogenic component secondary complex, a second immunogenic component antibody, labelled with a 15 reporter molecule capable of producing a detectable signal, is then added and incubated, allowing sufficient time for the formation of a tertiary complex. Any unreacted material is washed away, and the presence of bound labelled antibody is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal or may be quantitated by comparing with a control sample.
- 20 The present invention contemplates a range of variations to the subject assay including an assay for *L. intracellularis* antibodies using, for example, recombinant peptides, polypeptides or proteins from this organism.

The solid substrate is typically glass or a polymer, the most commonly used polymers being 25 cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs or microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing the molecule to the insoluble carrier.

By "reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, produces an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecule in this type of assay are either enzymes, fluorophores or 5 radionuclide containing molecules (i.e. radioisotopes). In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist which are readily available to one skilled in the art. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β -galactosidase and alkaline phosphatase, 10 amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. It is also possible to employ fluorogenic substrates, which yield a fluorescent product.

Alternatively, fluorescent compounds, such as fluorescein and rhodamine, may be chemically 15 coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off 20 the unbound reagent, the remaining ternary complex is then exposed to the light of the appropriate wavelength, the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed. It will 25 be readily apparent to the skilled technician how to vary the procedure to suit the required purpose.

A range of genetic diagnostic assays may be employed such as polymerase chain reaction (PCR) assays, hybridisation assays or protein truncation assays. All such assays are 30 contemplated in the present invention.

The present invention is further described by the following non-limiting Figures and/or Examples.

In the Figures:

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Figure 1 is a photographic representation showing Western analysis of *L. intracellularis* antigens recognised by vaccinated pigs. Track 1 (395) was probed with pig sera from a pig (395) that had been immunised three times with the formalin killed whole *L. intracellularis* vaccine. Track 2 to 5 (Y10, Y12, Y14, Y16) were probed with sera obtained from pigs Y1, 10 Y12, Y14 and Y16, respectively on day 0.

Figure 2 is a photographic representation of the small intestine obtained from pig Y1 on day 20.

15 **Figure 3** is a photographic representation of the small intestine obtained from pig Y2 on day 20.

Figure 4 is a photographic representation of the small intestine obtained from pig Y4 on day 20.

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The following single and three letter abbreviations are used for amino acid residues:

5 Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
10 Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
15 Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
20 Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
25 Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

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SUMMARY OF THE SEQUENCE IDENTITY NUMBERS

SEQ ID NO.	Description
5	1 Nucleotide sequence of GroEL
	2 Amino acid sequence of GroEL
	3 Nucleotide sequence of GroES
	4 Amino acid sequence of GroES
	5 Nucleotide sequence of <i>L. intracellularis</i> component
10	6 Nucleotide sequence of <i>L. intracellularis</i> component
	<u>7, and 8</u> Amino acid ^{sequences} sequence of SEQ ID NO:6
	<u>8</u> 9 Nucleotide sequence of <i>L. intracellularis</i> component
	9 10 Amino acid sequence of SEQ ID NO:8 (first coding sequence)
	10 11 Amino acid sequence of SEQ ID NO:8 (second coding sequence)
15	<u>11</u> 12 Nucleotide sequence of <i>L. intracellularis</i> component
	<u>12</u> 13 and 14 Amino acid ^{sequences} sequence of SEQ ID NO:11/12
	<u>13</u> 15 Nucleotide sequence of <i>L. intracellularis</i> component
	<u>14</u> 16-20 Amino acid ^{sequences} sequence of SEQ ID NO:13/15
	<u>15</u> 21 Nucleotide sequence of <i>L. intracellularis</i> component
20	<u>16</u> 22-27 Amino acid ^{sequences} sequence of SEQ ID NO:15/21
	<u>17</u> 28 Nucleotide sequence of <i>L. intracellularis</i> component
	<u>18</u> 29 Nucleotide sequence of <i>L. intracellularis</i> component
	<u>19</u> 30 Nucleotide sequence of <i>L. intracellularis</i> component
	<u>20</u> 31 Nucleotide sequence of <i>L. intracellularis</i> component
25	<u>21</u> 32 Nucleotide sequence of <i>L. intracellularis</i> component
	<u>22</u> 33 Nucleotide sequence of <i>L. intracellularis</i> component
	<u>23</u> 34 Nucleotide sequence of <i>L. intracellularis</i> component

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b' > EXAMPLE 1
SOURCES OF PIG TISSUE

Infected Pig Intestines

5 Sections of grossly thickened ilea were taken from pigs naturally or experimentally affected by PPE. The presence of *L. intracellularis* bacteria in the ilea was confirmed using immunofluorescent staining with specific monoclonal antibodies (10). An example of a suitable antibody is monoclonal antibody IG4 available from the University of Edinburgh, UK.

10

EXAMPLE 2

**ISOLATION OF *LAWSONIA INTRACELLULARIS* BACTERIA FROM THE
INFECTED PIG ILEUM**

Lawsonia intracellularis bacteria were extracted directly from lesions of PPE in pigs by 15 filtration and further purified over a Percoll (Pharmacia, Uppsala, Sweden) gradient. Infected ilea were collected from pigs and the presence of *L. intracellularis* was confirmed histologically before storage at -80°C. Sections of ileum were thawed and approximately 8g of infected mucosa were scraped from the intestinal wall. The mucosa was homogenised with 40 ml sterile phosphate buffered saline (PBS) on half speed for 10 s using a Sorvall omnimixer. This 20 suspension was centrifuged at 2000 xg for 4 minutes. The supernatant was discarded and the cell pellet was resuspended in 40 ml PBS and recentrifuged. This washing step was repeated twice. The cell pellet was then resuspended in 20 ml PBS and homogenised at full speed for one minute to release *L. intracellularis* bacteria.

25 This homogenate was centrifuged at 1000 xg for 4 minutes giving a pellet containing a crude mixture of homogenised epithelial cells and intestinal bacteria. The supernatant was filtered using filters with pore sized 3 μm , 1.2 μm and 0.8 μm (Millipore Corporation, MA, USA). The filtrate was centrifuged at 8000 xg for 30 minutes, resulting in a small pellet of *L. intracellularis* bacteria. The *L. intracellularis* bacteria were further purified using a 45% self forming percoll 30 gradient as follows: 2 mls of the bacterial preparation was mixed by inversion into 30 mls of

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a 45% self forming Percoll (Pharmacia LKB, Uppsala, Sweden) gradient (45% v/v of Percoll, 150 mM NaCl). The gradients were centrifuged in a Sorval centrifuge using the SS34 rotor, at 20,000rpm for 30 minutes at 4°C. Usually a number of bands form within the gradient. The band (usually located approx. 10-20mm from the base of the tube) containing the *L. 5 intracellularis* bacteria was collected and the volume made up to 16 mls with PBS. The solution was then centrifuged for 15 minutes at 8000rpm. The resultant pellet was washed with PBS before being resuspended in a final volume of approximately one ml.

EXAMPLE 3

10 PURIFICATION OF *LAWSONIA INTRACELLULARIS* GENOMIC DNA

Genomic DNA was extracted from percoll-gradient purified *Lawsonia intracellularis* bacteria, recovered from infected pig ilea scrapings (Example 2), by the methods described by Anderson *et al* (11) & Sambrook *et al* (12).

15 EXAMPLE 4

IMMUNOSCREENING OF GENOMIC LIBRARIES

20 A lambda ZAP II *L. intracellularis* genomic library was plated on a lawn of *Escherichia coli* XLI-Blue (23) cells at a density of 2,000 plaque-forming units (pfu) per 150 mm L-broth agar plate. The library was screened with a rabbit anti- *L. intracellularis* sera using the method described in the Protoblot Technical Manual (Promega, WI, USA). Filters were blocked in a buffer containing 10mM Tris HCl, pH8.0, 150mM NaCl, 0.05% Tween 20, 1% w/w gelatin. Positive plaques identified in a primary screen were picked, replated at a lower density and rescreened until individual positive plaques were identified.

25

EXAMPLE 5

ISOLATION AND SEQUENCING OF cDNA INSERTS

30 Phagemid DNA from positive λZAP II phage clones was isolated by excision *in vivo* of the pBluescript phagemid under the conditions recommended by Stratagene (CA, USA). Plasmid

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DNA was either extracted by the method of Birnboim and Doly and the cDNA inserts sequenced by the chain termination method (21), or by the PEG-precipitation method and cycle-sequenced by the dye-terminator method, as recommended by the manufacturer (Applied Biosystems).

5

EXAMPLE 6

ANTISERA

10 Antisera to *L. intracellularis* bacteria were raised in rabbits and pigs. Rabbits were injected intramuscularly with a preparation of Percoll gradient-purified *L. intracellularis* bacteria mixed with a double-emulsion made by processing with oil adjuvant (Freund's incomplete adjuvant, CSL Limited, Melbourne, Australia), and then with Tween 80 enhancer. Two 3 ml injections, containing 9 mg protein, were given four weeks apart. Blood samples were collected from the marginal ear vein prior to immunisation and two weeks following the second injection.

15

15 A 6-week old pig (395) was hyperimmunised by intramuscular injection of Percoll gradient-purified *L. intracellularis* bacteria prepared with Freund's incomplete adjuvant as for the rabbit. Three injections of the prepared antigen were administered four weeks apart, and blood was collected from the jugular vein two weeks following the final injection. Diluted pig sera (1 ml, 20 1 in 200) were pre-absorbed with 100 μ l *E. coli* DH5 α (24) lysate for 1 h at room temperature with gentle mixing. The lysate was prepared by freeze-thawing a suspension of *E. coli* in PBS.

EXAMPLE 7

SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL

25

ELECTROPHORESIS (SDS-PAGE)

Protein samples were resuspended in 50 μ l of sample buffer (62.4 mM HCl, 2% w/v SDS, 10% v/v glycerol, 5% v/v 20 mercaptoethanol, 0.002% bromophenol blue, pH 6.8) and heated to 95 °C for 5 minutes before separating solubilised proteins electrophoretically on a 0.1% w/v 30 SDS-12% w/v PAGE vertical slab gel (13).

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EXAMPLE 8

WESTERN BLOTTING

5 *Proteins were electrophoretically transferred to Immobilon-P (Millipore Corporation, MA, USA) membranes in a Trans-Blot Cell (BioRad, CA, USA) at 100 V for 1 h in a buffer containing CAPS (3-[Cyclohexylamino]-1-propanesulfonic acid, pH 11, Sigma, MI, USA) and 10% v/v methanol. The membranes were then blocked with 5% w/v Blotto (Diploma skim milk powder, Melbourne, Australia) in PBS for 30 min at room temperature with gentle rocking. The filters were then transferred to antisera diluted in 5% w/v Blotto, PBS. Pre-10 absorbed pig antisera was diluted 1 in 200. The filters were incubated in pig antisera for 1 h followed by washing three times in PBST.*

15 *HRP conjugated anti-swine immunoglobulins (DAKO, CA, USA) were applied at a dilution of 1:2000. Enhanced Chemiluminescence (ECL, Amersham, IL, USA) was used to discriminate *L. intracellularis* proteins. Prior to ECL detection, blots were washed three times for 7 minutes each. The filters were exposed to autoradiographic film (Agfa, NJ, USA) for less than 1 minute before developing.*

EXAMPLE 9

IDENTIFICATION OF GroEL AND GroES

20 Clones found to be positive according to the immunoscreening method described in Example 4 were sequenced using the protocol detailed in Example 5. One clone isolated represented the GroEL protein. The nucleotide sequence and corresponding amino acid sequence of GroEL 25 are shown in SEQ ID NO:1 and SEQ ID NO:2. Another clone isolated represented the GroES protein. The nucleotide sequence of GroES and corresponding amino acid sequence are shown in SEQ ID NO:3 and SEQ ID NO:4.

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EXAMPLE 10

IMMUNOFLORESCENT DETECTION OF *LAWSONIA INTRACELLULARIS* BACTERIA IN PIG FAECES

5 Faecal swabs of pigs were taken using a cotton tipped swab and then the sample was smeared onto a glass slide. After allowing ten minutes for air drying the smears were heat fixed by heating to 60°C for approximately 10 seconds. The slides were then rinsed in PBS. An amount of 30 μ l of a 1/200 dilution of a mouse ascites containing IgG monoclonal antibody (see Example 1) was added, a glass cover slip applied, and the slides were incubated at room 10 temperature for 40 minutes. The cover slip was removed and the slides were washed (PBST for 7 minutes, three times). An amount of 30 μ l of a 1/40 dilution of a FITC conjugated anti-mouse antiserum (Silenus, Melbourne Australia) was added, a glass cover slip applied and the slides were incubated at room temperature for 40 minutes. The cover slip was removed and the slides were washed (PBST for 7 minutes X3). The slides were given a final rinse in PBS. A drop of 15 10% v/v glycerol PBS was added and a glass cover slip applied. The fluorescent bacteria were visualised under highpower (X1200) at 340 nm using a Lietz laborlux S microscope. Twenty fields were counted and the results (see Table 1) were expressed as the average number of *L. intracellularis* bacteria per high powered field.

20

EXAMPLE 11

FORMALIN-KILLED *L. INTRACELLULARIS* VACCINE

1 The percoll gradient purified bacterial *L. intracellularis* pellet was resuspended in 1 ml of 1% formalin in saline and incubated overnight at 4°C. The percoll gradient-purified *L. intracellularis* bacteria was then mixed into a double-emulsion made by processing with oil adjuvant (Freund's incomplete adjuvant, Commonwealth Serum Laboratories, Melbourne, Australia), and then with Tween 80 enhancer.

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EXAMPLE 12
VACCINATION PROTOCOL

5 Twelve weaned pigs (Landrace crossed with Large White) were sourced from a Pig Improvement Company piggery and treated with Neo-Terramycin (0.25 g/kilo) for 5 days. Seven days later (day -40) pigs Y10, Y12, Y14 and Y16 were vaccinated as described. Pigs Y3, Y11 and Y13 were treated for abscess with long acting terramycin on day -34.

10 The twelve pigs were divided into three groups and treated as follows:

Group 1 Infected Controls
Four pigs (Ear Tag No Y1-Y4) were housed with vaccinated pigs.

15 *Group 2 Whole Bacteria Vaccine*
Four pigs (Ear Tag No. Y10, Y12, Y14 and Y16) were immunised with 0.5 ml formalin killed *L. intracellularis* bacteria emulsified in 0.5 ml of PBS/Freunds incomplete adjuvant on days -33 and -12.

20 *Group 3 Uninfected Controls*
Four pigs (Ear Tag No. Y9, Y11, Y13 and Y15) received no treatments and were housed in a separate area from the vaccinated pigs and infected control pigs.

EXAMPLE 13
ORAL CHALLENGES OF INFECTED PIGS

Infected ilea were collected from pigs as described in Example 1 and the presence of *L. intracellularis* was confirmed histologically before storage at -80°C. Sections of ileum were thawed and approximately 150g of infected mucosa was scraped from the intestinal wall. The 30 mucosa was homogenised with an equal volume of sterile PBS on half speed for 20 s using a

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Sorvall ominiimixer. This suspension was diluted two fold with sterile PBS to form the challenge suspension.

On day 0 each pig from Groups 1 and 2 was dosed with a 5% w/v with Na Bicarbonate solution 5 (10 ml/kg) followed by 30 ml of the challenge suspension. This was repeated on day 1 and day 2.

From day 11 onwards, the number of *L. intracellularis* bacteria in each pig's faeces was monitored by immunoflorescence. Pigs were monitored for signs of disease and shedding of 10 *L. intracellularis* bacteria. Pigs shedding greater than 100 bacteria per high powered field and scouring were killed for ethical reasons.

On day 22 the surviving pigs were humanely killed and the small intestines were recovered. Two sections of small intestine were removed 5 cms and 17 cms proximally from the ileocaecal 15 junction. These sections were fixed in 10% v/v formalin, wax embedded and sections were sent to an independent veterinary pathologist for analysis.

EXAMPLE 14

LAWSONIA INTRACELLULARIS PROTEINS RECOGNISED BY VACCINATED PIGS

20

Antibodies raised by pigs to *L. intracellularis* proteins post vaccination were analysed by Western blotting followed by ECL (Amersham, IL, USA) detection as described in Example 8. The results are shown in Figure 1. Vaccinated pigs produce antibodies to a range of *L. intracellularis* proteins. The most immunodominant proteins recognised are approximately 62.7 25 Kda, 58.7 Kda, 57.2 Kda, 44 Kda, 36.7 Kda and two smears from 24-26 Kda and 22-23.5 Kda. Minor immunoreactive bands had approximately the following molecular weights 67 Kda, 52.5 Kda, 50.5 Kda, 50 Kda, 48.2 KDa, 47.9 Kda, 44.7 Kda, 43.5 Kda, 42.5 Kda, 41.5 Kda, 40.5 Kda, 39 Kda, 35.3 Kda, 17 Kda, 15.5 Kda, 12 Kda and 7 Kda. The molecular weight of the proteins recognised will vary by up to 5% depending on the method used for estimation.

30

EXAMPLE 15**SHEDDING OF *L. INTRACELLULARIS* BACTERIA BY PIGS DURING TRIAL**

Three of the pigs from Group 1 (Infected control) in Example No. 12 (Y1, Y2 and Y4) shed 5 greater than 100 *L. intracellularis* bacteria per high powered field in their faeces by day 19 post oral challenge (Table 1). Two of these pig (Y2 and Y4) had a bloody scour. All three pigs were 10 humanely killed on day 20. Y3 shed low levels of *L. intracellularis* bacteria during the course of the infection trial. Maximal bacterial shedding for Y3 was 16 bacteria per high powered field.

10

All pigs in group 3 vaccinated with whole bacteria as set out in Example 12, never shed more than 3 *L. intracellularis* bacteria per high powered field. Vaccination with the formalin killed *L. intracellularis* vaccine reduced total bacterial shedding of *L. intracellularis* bacteria by 15 vaccinated pigs by 98.5% when compared with group 1 pigs.

15

None of the group 3 pigs (uninfected controls) shed any *L. intracellularis* bacteria during the course of the trial.

The results of shedding of *L. intracellularis* bacteria per pig are shown in Table 1.

20

EXAMPLE 16
GROSS PATHOLOGY FOR TRIAL A***Group 1 Infected Controls***

25 Y1 Approximately 5 cm of terminal ileum was grossly thickened. No other signs of PPE were evident macroscopically. Findings are consist with intestinal adenomatosis (See Figure 2).

Y2 The intestine was found to be grossly thickened and the serosa had the characteristic cerebriform f rms (Figure 3). Over 2.5 metres of the intestine was involved. The lumen 30 of the intestine was found to contain fresh blood and fibrinous casts were evident.

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Proliferative haemorrhagic enteropathy.

Y3 No gross signs of PPE were evident.

Y4 The intestine was found to have necrotic enteritis (Figure 4). The mucosal surface was replaced with a fibrinous pseudomembrane. Oedema of the mesentery was clearly evident. Over 2.0 meters of intestine was involved.

Group 2 Whole L. intracellularis cell vaccine

Y10 No gross signs of PPE.

Y12 No gross signs of PPE.

10 Y14 No gross signs of PPE.

Y16 No gross signs of PPE.

Group 3 Uninfected controls

Y9 No gross signs of PPE.

15 Y11 No gross signs of PPE.

Y13 No gross signs of PPE.

Y15 No gross signs of PPE.

EXAMPLE 17

20 **HISTOPATHOLOGY REPORT FOR TRIAL**

Reports are based on established histopathological descriptions in Jubb *et al* (20).

Group 1 Infected control group

25 Y1 Numerous microfocal/confluent lesions of Porcine Intestinal Adenomatosis (PIA) are associated with Peyer's Patches.

Y2 Serious generalised (annular) lesions of Porcine Intestinal Adenomatosis.

Y3 No conclusive evidence of PIA. Sparse microfocal lesions suggestive of a non-specific mild reactive (reparational) hyperplasia (rather than an adenomatosis).

30 Y4 Severe generalised (annular) lesions of PIA.

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Group 2 Whole L. intracellularis cell vaccine

Y10 No conclusive evidence of PIA.
Y12 No conclusive evidence of PIA.
5 Y14 No conclusive evidence of PIA.
Y16 No conclusive evidence of PIA. Possible single microfocus of PIA is associated with Payers Patch.

Group 3 Uninfected controls

10 Y11 No conclusive evidence of PIA.
Y9 No conclusive evidence of PIA.
Y13 Intestine was not recovered since pig was killed due to lameness at day 15.
Y15 Diagnosis not possible because of the poor quality sections.

15

EXAMPLE 18

**IMMUNOSCREENING OF A *L. INTRACELLULARIS* LIBRARY USING
EXPERIMENTAL SERA FROM VACCINATED PIGS**

20 *L. intracellularis* genomic DNA was purified as described in Example 3. The DNA was partially digested with the restriction endonuclease *Sau3A* (Promega) and ligated into Lambda ZAP II Express (Stratagene). The lambda library was plated on a lawn of *E. coli* XLI-Blue cells at a density of 10,000 pfu per 150 Mm L-broth agar plate. The library was screened, as described in Example 4, with sera from Y12. The pig Y12 was immunised with formalin killed 25 *L. intracellularis*, as described in Example 11 & 12. Vaccinated pigs produced antibodies to a range of *L. intracellularis* proteins, as described in Example 14. A number of phage clones expressing *L. intracellularis* proteins were identified.

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EXAMPLE 19

ANALYSIS OF *L. INTRACELLULARIS* EXPRESSING PHAGE CLONES

5 Phagemid DNA from positive λ ZAP II Express phage clones was isolated by *in vivo* excision, by the conditions recommended by the manufacturer (Stratagene). Plasmid DNA, for restriction analysis was extracted by alkaline-lysis, as described by Sambrook *et al* (12), and for automated sequencing, using the High Pure Plasmid Kit, as recommended by the manufacturer (Boehringer Mannheim). DNA sequencing of inserts was performed by the Dye-terminator method of automated sequencing (ABI Biosystems). The sequences identified are set out in SEQ ID NOS: 5-23 (see Example 20).

EXAMPLE 20

IDENTIFICATION OF *L. INTRACELLULARIS* COMPONENTS

15 Sequence similarity of the DNA molecules encoding putative vaccine candidates identified from Example 18 and 19, was identified using BLAST (27). Nucleotide sequence SEQ ID NO:6 and its corresponding amino acid sequence SEQ ID NO:7 have sequence similarity to flagellar basal body rod protein. SEQ ID NO:8 (nucleotide) and SEQ ID NOS:9 and 10 (amino acid) have sequence similarity to autolysin. SEQ ID NO:11 (nucleotide) and SEQ ID NO:12 (amino acid) show sequence similarity to S-adenosylmethionine: tRNA ribosyltransferase-isomerase (queuosine biosynthesis protein queA).

15 NOS:16-20
B SEQ ID NO:13 (nucleotide) and SEQ ID NO:14 (amino acid) show sequence similarity to 21 enoyl-(acyl-carrier-protein) reductase. SEQ ID NO:15 (nucleotide) and SEQ ID NO:16 (amino acid) show sequence similarity to a glucarate transporter. Other nucleotide sequences 22-27 encoding putative vaccine candidates are SEQ ID NO:5, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23.

30 Those skilled in the art will appreciate that the invention described herein is susceptible to

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variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more
5 of said steps or features.

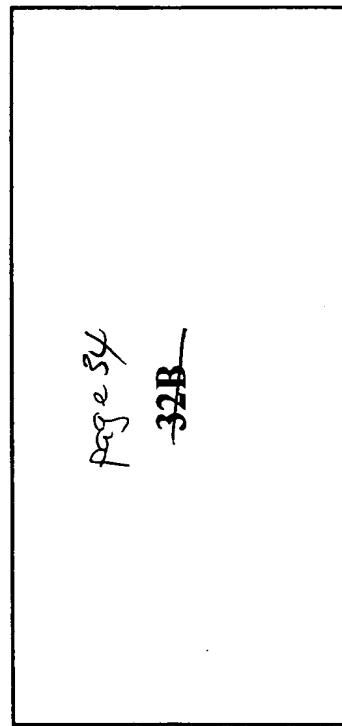
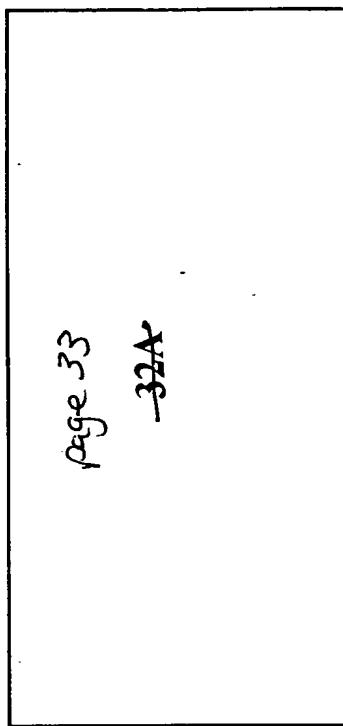
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TABLE 1



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TABLE 1

Vaccination	Challenge
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	Day -40	Day -33	Day -26	Day -12	Day 0	Day 1	Day 2	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22
1 infected controls																			
	1+	1+	0	0															
2 infected controls																			
	0	1+	1+	1+															
3 infected controls																			
	0	0	0	0	0	0	0												
4 infected controls																			
	1+	0	0	10+	0														
10 whole bugs																			
	0	0	0	0	0	0	1+	1+	1+	0	0	0	0	0	0	0	0	0	
	1 ml killed whole cell																		

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12 whole bugs	1 ml killed whole cell 1 ml killed whole cell	1+	0	0	0	2+	0	0	0	0	0	0
14 whole bugs	1 ml killed whole cell 1 ml killed whole cell	0	0	0	0	1+	0	<1	<1	0	0	0
16 whole bugs	1 ml killed whole cell 1 ml killed whole cell	0	0	0	0	0	0	3	<1	0	0	0
9 Uninfected controls		0	0	0	0	0	0	0	0	0	0	0
11 Uninfected controls		0	0	0	0	0	0	0	0	0	0	0
13 Uninfected controls		0	0	0	0	0	0	0	0	0	0	0
15 Uninfected controls		0	0	0	0	0	0	0	0	0	0	0

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